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# MOLECULAR REGULATION OF SOMATOSENSORY NEURON DEVELOPMENT

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On the front cover: Whole-mount immunofluorescence of developing nerves in embryonic mouse forelimbs.
Credits: Saida Hadjab
On the back cover: Drawing depicting regulatory mechanisms of neuronal specification.
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# MOLECULAR REGULATION OF SOMATOSENSORY NEURON DEVELOPMENT

## THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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Dedicated to my dearest family

献给我最亲爱的家人

# ABSTRACT

The somatosensory system of vertebrates perceives and transmits a variety of information from both external and internal environments to the central nervous system where an integrated response is established leading to adaptive outcome. Specific classes of sensory neurons convey the information consisting of touch, muscle stretch, temperature, itch, and pain. Each type of sensory neuron expresses a group of specific markers or proteins in order to perform a specialized function. However, the mechanisms that ensure the acquisition of various molecular traits by somatosensory neurons during development is still not fully understood. This doctoral thesis explores several early developmental events for different types of somatosensory neurons at molecular and cellular levels in order to reduce the gap of knowledge in this field.

In Paper I and II, we investigated the neuronal specification of nociceptive neurons, which were derived from specific waves of neurogenesis. We found that PRDM12, an epigenetic regulator, was necessary for the entire nociceptive lineage to develop. In the absence of PRDM12, neural crest precursors failed to generate all of the nociceptive neurons. We also found that the key transcription factor RUNX1, which plays an important role in the diversification of nociceptive neurons, was induced by factors released by early born neurons, emphasizing the important influence of the environment created by early postmitotic neurons on the fate of later born neurons.

In Paper III, we proposed a new cell selection model in the early cell death of sensory neurons using the proprioceptive neurons population as a model system. The canonical neurotrophic theory suggests similarity of neurons when competing for target-derived neurotrophins for their survival. However, our data showed that early proprioceptive neurons exhibit a molecular heterogeneity code leading to different capacities to survive already before the cell death period. Further, this capacity was intrinsically regulated by the transcription factor RUNX3 whose expression was defined by the surrounding morphogen retinoic acid.

Finally, in Paper IV, we showed that the transcription factor RUNX3 controls the axonal growth rate of developing sensory neurons in a strict temporal and spatial manner. Taking advantage of both chicken embryos and mouse genetics, we observed that the difference in peripheral nerve growth at different axial levels was encoded by RUNX3 expression.

In summary, the data collected in this thesis describes several new insights into the molecular regulation during the step-wise development of somatosensory neurons, including neurogenesis, neuronal specification, early cell death, and axonal growth. This knowledge will help us to the better understanding of the development of the somatosensory system as well as provide new knowledge that might help improving approaches of treatment for patients with somatosensory disorders such as congenital insensitivity to pain.

# LIST OF SCIENTIFIC PAPERS

I. **Wang Y***, Bartesaghi L*, Fontanet P, Wanderoy S, Berger F, Wu H, Akkuratova N, Bouçanova F, Médard JJ, Landy MA, Zhang M, Harrer P, Stendel C, Stucka R, Dusl M, Kastriti ME, Croci L, Lai HC, Consalez GG, Pattyn A, Ernfors P, Senderek J, Adameyko I, Chrast R, Hadjab S, Lallemend F. PRDM12 is required for initiation of the nociceptive neuron lineage during neurogenesis.
Under revision in *Cell Reports*.

II. Hadjab S, Franck MC, **Wang Y**, Sterzenbach U, Sharma A, Ernfors P, Lallemend F. (2013). A local source of FGF initiates development of the unmyelinated lineage of sensory neurons.
*Journal of Neuroscience*, 2013 Nov 6; 33(45):17656-66.

III. **Wang Y***, Hadjab S*, Wu H, Codeluppi S, Sharma A, Xue-Franzén Y, Niederreither K, Silva FD, Comai G, Petitpré C, Agirman G, Palumberi D, Linnarsson S, Moqrich A, Schedl A, Lallemend F.
A cell fitness selection model for neuronal survival during the development.
Under revision in *Nature Communications*.

IV. Lallemend F, Sterzenbach U, Hadjab-Lallemend S, Aquino JB, Castelo-Branco G, Sinha I, Villaescusa JC, Levanon D, **Wang Y**, Franck MC, Kharchenko O, Adameyko I, Linnarsson S, Groner Y, Turner E, Ernfors P. Positional differences of axon growth rates between sensory neurons encoded by Runx3.
*EMBO Journal*, 2012 Sep 12; 31(18):3718-29.

*These authors contributed equally

## Papers not included in this thesis:

I. **Wang Y**, Hadjab S, Lallemend F. A muscle-type specific function of RUNX3 for the construction of sensory-motor circuit.
*Manuscript*.

II. Petitpré C, Wu H, Sharma A, Tokarska A, Fontanet P, **Wang Y**, Helmbacher F, Yackle K, Silberberg G, Hadjab S, Lallemend F. Neuronal heterogeneity and stereotyped connectivity in the auditory afferent system.
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III. **Wang Y***, Fisahn A*, Sinha I*, Nguyen DP, Sterzenbach U, Lallemend F, Hadjab S. Hippocampal transcriptome profile of persistent memory rescue in a mouse model of THRA1 mutation-mediated resistance to thyroid hormone. *Scientific Reports*, 2016 Jan 8; 6:18617.

IV. Vandenbosch R, Chocholova E, Robe PA, **Wang Y**, Lambert C, Moonen G, Lallemend F, Malgrange B, Hadjab S. A role for the canonical nuclear factor-κB pathway in coupling neurotrophin-induced differential survival of developing spiral ganglion neurons.
*Frontiers in Cellular Neuroscience*, 2013 Nov 28; 7:242.

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| BDNF | Brain-Derived Neurotrophic Factor |
| BMP | Bone Morphogenetic Protein |
| CAM | Cell Adhesion Molecule |
| CGRP | Calcitonin Gene-Related Peptide |
| CI | Cortical Interneurons |
| CIP | Congenital Insensitivity to Pain |
| CNS | Central Nervous System |
| DRG | Dorsal Root Ganglion |
| E | Embryonic Day |
| eGFP | Enhanced Green Fluorescent Protein |
| eTRKA | Early TRKA |
| FGF | Fibroblast growth factor |
| FGFR1 | Fibroblast Growth Factor Receptor 1 |
| GDNF | Glia cell-Derived Neurotrophic Factor |
| GTO | Golgi Tendon Organ |
| IB4 | Isolectin B4 |
| IGF | Insulin-like Growth Factor |
| LTMR | Low-Threshold Mechanoreceptor |
| lTRKA | Late TRKA |
| MN | Motor Neuron |
| MS | Muscle Spindle |
| MSN | Mechanoreceptive Sensory Neuron |
| NCC | Neural Crest Cell |
| NGF | Nerve Growth Factor |
| NGN | Neurogenin |
| NSN | Nociceptive Sensory Neurons |
| NT3 | Neurotrophin-3 |
| NF200 | Neurofilament 200 kDA |
| P | Postnatal Day |
| PCD | Programmed Cell Death |

| | |
|---|---|
| PRDM12 | PRDI-BF1 and RIZ homology domain-containing protein 12 |
| PSN | Proprioceptive Sensory Neuron |
| PV | Parvalbumin |
| RA | Retinoic Acid |
| RET | REarranged during Transfection |
| RUNX | Runt-related Transcription Factor |
| SOX10 | Sex Determining Region Y |
| TRK | Tropomyosin Receptor Kinase |
| TH | Tyrosine Hydroxylase |
| WNT | Wingless/Integrated |

# 1 INTRODUCTION

The somatosensory system is a complex system that allows for interpretation of external stimuli as well as internal changes in the body. This system covers the entire body through the extension of nerves. In the periphery, the endings of these nerves contain structures known as sensory receptors, which can be subdivided into at least five categories depending on the type of information that they sense: proprioceptors (position of the body in space), mechanoreceptors (vibration and touch), nociceptors (pain), pruriceptors (itch) and thermoreceptors (heat and cold). These different receptors convey their corresponding modality of sensory information from the periphery to the central nervous system (CNS) (Kandel et al., 2012).

The classic pathway of a somatosensory circuit is mainly comprised of three relay neurons that are located at different levels from the periphery to the cerebral cortex (see Figure 1). The primary relay neuron is pseudo-unipolar, whose cell body is located either in the dorsal root ganglia (DRG), which are structures organized in pairs alongside the spinal cord, or in the trigeminal ganglia if sensation is from the head or neck. DRG neurons receive different somatosensory information from the peripheral receptors after which it conveys the information to the secondary neurons in the CNS. The second relay neuron has its soma either in the spinal cord or brainstem nucleus. Its ascending axon decussates to the opposite side of the body either at the level of the spinal cord or the brainstem. The second neuron afferent either synapses with a third relay neuron in the spinal cord or ascend to synapse in the thalamus that relay sensory impulses to the somatosensory cortex where all the relayed information are integrated and a response can be initiated (Saladin, 2004).

Most of the information collected from the peripheral nerves passes through the above-mentioned pathway to cortex for processing. However, some stimuli require an acute response in the shape of a reflex, without the necessity of involving brain consciousness. The circuitry then involved is local typical reflex loop is composed of two parts: the afferent that transmits information from the peripheral receptor to the CNS and the efferent, which sends out information leading to a response from the spinal cord or brainstem to the periphery. One classic example is the muscle stretch reflex, initiated when the receptor within the skeletal muscle is rapidly stretched. Here the afferent proprioceptive neuron transduces the signal to particular set of motor neurons within ventral horn of the spinal cord. In return, the motor neurons send out the signal through the efferent axons to the same muscle, leading to contraction (Boron and Boulpaep, 2012).

While the general structure of the somatosensory system has been known for more than a century, its development, especially of the diversity of its components, is still not fully elucidated. Many questions remain unanswered, including how the specification and integration of different sensory neuron subgroups into functional neuronal circuits throughout development occurs. This thesis focuses on the development of DRG neurons as a model to study the mechanisms underneath different steps of neuronal development, ranging from neurogenesis to axonal growth.

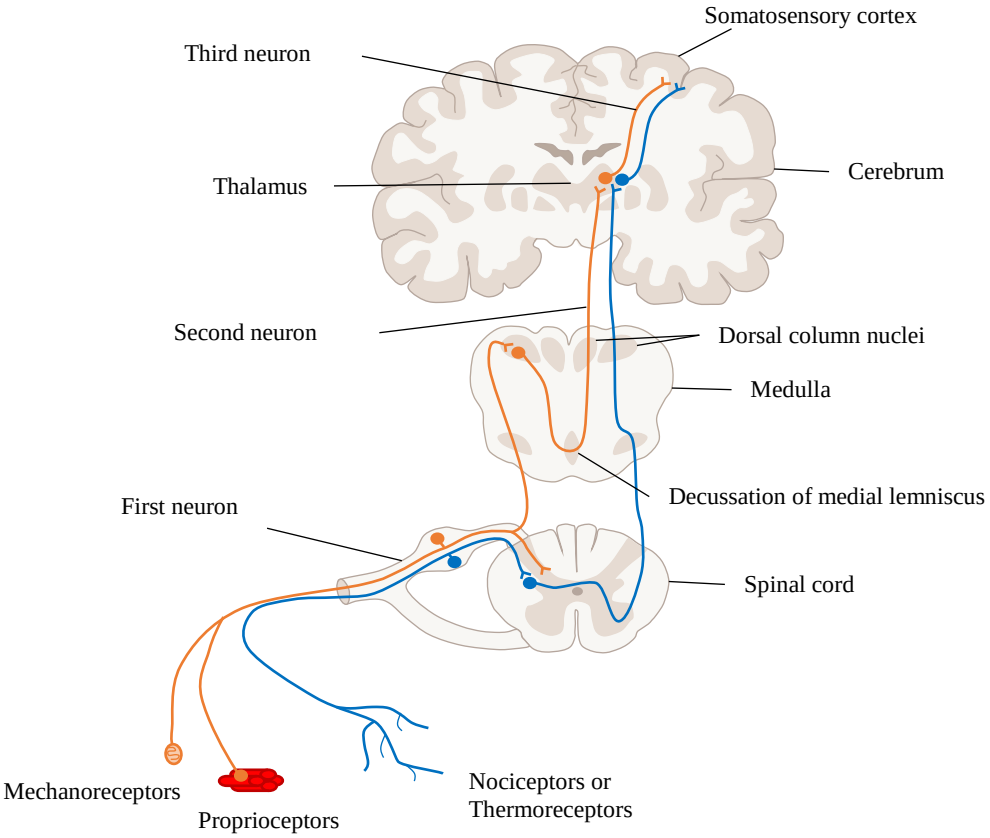


**Figure 1. Anatomical features of the spinal somatosensory system pathway.**
*The orange line shows the pathway mechanoreceptors or proprioceptors whereas blue line shows the pathway of the nociceptors or thermoreceptors pathway (Modified from Ganong's Review of Medical Physiology, 23rd ed.2009).*

## 1.1 NEUROGENESIS

DRG neurons derive from a group of multipotent stem cells called neural crest cells (NCCs). At the end of the neurulation process, NCCs position themselves dorsally of the early neural tube (Basch et al., 2006). NCCs maintain their multipotency and proliferative capacities as they express the sex determining region Y box 10 (SOX10) gene which will be repressed at the beginning of neurogenesis (Kim et al., 2003).

In the mouse DRG, neurogenesis first occurs at around mouse embryonic day (E) 9.5 and ends at around E13.5. As observed by time-lapse confocal microscopy, NCCs migrate alongside the neural tube in a chain-like shape to coalesce into DRG between E9.5 and E10.5 (Kasemeier-Kulesa et al., 2005; Serbedzija et al., 1990). Already during migration, some of the NCCs exit cell cycle to commit to neuronal fate and lose SOX10 expression (Marmigère and Ernfors, 2007).

Wingless/Integrated (WNT) and bone morphogenetic protein (BMP) related signaling pathways seem to play an important role in directing the NCCs towards sensory neuronal lineages. Both protein families are expressed in the dorsal neural tube during the NCCs migration. Disruption of WNT signaling results in a failure of the NCCs to differentiate into DRG neurons during migration (Hari et al., 2002) while its activation in NCCs can promote an ectopic formation of DRG neurons in anterior regions of the embryos (Lee et al., 2004). In contrast, BMP signaling antagonizes the sensory neuron fate induced by the activity of WNT signaling. The maintenance of NCCs requires the activity of both growth factors (Kléber et al., 2005).

### 1.1.1 Three Waves of neurogenesis

The neurogenesis of DRG neurons follows three consecutive waves. The first group of post mitotic cells generate medium to large myelinated neurons that will express the neurofilament 200 kDa (NF200) protein at birth, whereas the later-born group of neurons generate small unmyelinated neurons that express peripherin (Ferri et al., 1990; Lawson and Biscoe, 1979). The timing of these two waves of neurogenesis corresponds to the consecutive expression of two neurogenic transcription factors in $SOX10^+$ precursors: neurogenin (NGN) 2 and NGN1. NGN2 drives the first wave of neurogenesis in which most of the neurons express either the neurotrophic factor receptors tropomyosin-receptor-kinase B (TRKB) or TRKC, while only a few of them express TRKA (early TRKA, eTRKA) or the RET (REarranged during Transfection) receptors. NGN1 drives the second wave of neurogenesis in which all neurons express TRKA (late TRKA, lTRKA). TRKA, TRKB, TRKC and RET bind to their ligands nerve growth factor (NGF), brain-derived growth factor (BDNF), neurotrophin-3 (NT3) and glial cell-derived growth factor (GDNF), respectively. These receptors are important for the subsequent events; the survival of each type of DRG neurons, the innervation of their peripheral targets and finally, the receptors expression is important to acquire specific expression of molecular traits that further define their characteristics (Lallemend and Ernfors, 2012). The third wave of neurogenesis, less studied, come from clusters of NCCs called

boundary cap cells that give rise to few TRKA$^{+}$ neurons and satellite glial cells in the DRG (Marol et al., 2004).

The neurogenins are necessary for the development of all DRG neurons. Indeed, in the double knockout mice for both *Ngn1* and *Ngn2* genes, DRG neurons do not form at all (Ma et al., 1999). Interestingly, in the absence of NGN2, NGN1 can compensate for the generation of the early neurons (NGN2 dependent) indicating that NGN1 and NGN2 are not necessary for the generation of specific subclasses of sensory neurons but are essential for time-wise neurogenesis.

## 1.2 NEURONAL DIVERSIFICATION

Soon after neurogenesis, at least five different lineages of DRG neurons emerges with expression of different sets of receptors and transcription factors. Three of those lineages represent the low-threshold mechanoreceptors (A-LTMRs) and are defined by the expression of RET/MAFA (mechanoreceptors), TRKB/SHOX2 (mechanoreceptors), TRKC/RUNX3 (proprioceptors). The remaining two of the lineages represent the nociceptive fate and are defined by the expression of TRKA (early TRKA, eTRKA population, Aδ fibers) or TRKA/RUNX1 (late TRKA, lTRKA population, C-fibers) (Lallemend and Ernfors, 2012). The homeobox transcription factor BRN3A and ISLET1 (ISL1) are believed to collectively play a role in the early neuronal specification. As soon as they exit the cell cycle, all presumptive DRG neurons express both BRN3A and ISL1 (Lanier et al., 2010; Sun et al., 2008). Interestingly, neurogenesis still occurs in *Brn3a*/*Islet1* double knockout mice, but the DRG neurons do not express any DRG neuron markers including TRKA, TRKB, TRKC, as well as the runt-family transcription factors RUNX1 and RUNX3, which regulate the fate of most TRKA and TRKC neurons respectively (Dykes et al., 2011; Sun et al., 2008). When analyzed separately, BRN3A is necessary for the early specification of the TRKC and TRKB lineages while ISL1 is important for that of the TRKA, RET and TRKB lineages.

While the expression of TRK and RET receptors can be used to label specific lineages during embryogenesis, genetic tracing using *TrkC*$^{Cre}$;*Rosa26*$^{GFP}$ reporter mouse line has shown that TRKC is also expressed in early TRKA, TRKB and in RET populations at a certain time point soon after neurogenesis (Hadjab et al., 2013) revealed by the expression of the GFP reporter in all those neuronal population. Similarly, TRKA is expressed in all DRG neurons during neurogenesis. Furthermore, a lot of evidence show that early immature DRG neurons share the expression of several other markers at early stages, like SHOX2 or RUNX3, after which those markers become segregated amongst DRG neuron type as they acquire the

expression of specific transcription factor sets to further diversify (Abdo et al., 2011; Bachy et al., 2011; Kramer et al., 2006). This is a classic trait of neuronal diversification where early born neurons have overlapping subtype identities. As development proceeds, molecular interactions occur within the cell, neurons differentiate and subtypes become more distinct from each other (Greig et al., 2013). In principle, activities and specificity of transcription factors in postmitotic neurons determine specific cell fates, by enhancing the molecular traits of the corresponding cell type and repressing those of other cell types (Lallemend and Ernfors, 2012). Figure 2 illustrates this process, showing how the activity of particular transcription factors segregates the $TRKC^+$ population (presumptive proprioceptive sensory neurons, PSNs) from the $TRKB^+$ population (future mechanosensory neurons innervating the skin). The transcription factor RUNX3 is expressed shortly after the expression of TRKC in the presumptive PSNs to maintain TRKC level and suppress the expression of TRKB and SHOX2 (Abdo et al., 2011; Kramer et al., 2006). On the other hand, SHOX2 is necessary for the expression of TRKB and suppresses the RUNX3 expression in these $TRKB^+$ mechanosensory neurons (Abdo et al., 2011). Thus, this cross-regulatory network of transcription factors segregates these two populations by E11.5. Although the specification of early TRKA and RET neurons is not well understood, similar mechanisms of interaction between transcription factors are presumed to operate to specify these two neuron types. For example, within the LTMR population that is $RET^+/MAFA^+$, the transcription factors MAFA/c-MAF are necessary to maintain the expression of RET which then drive the LTMR fate (Bourane et al., 2009; Wende et al., 2012).

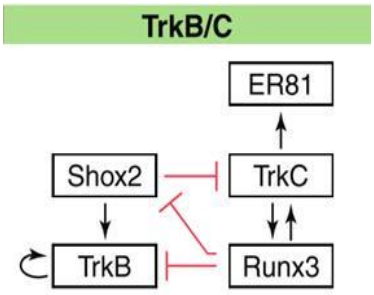


**Figure 2. Gene regulatory networks during specification of PSNs neurons.**
*Molecular interactions regulating diversification between $TRKC^+$ and $TRKB^+$ population. Arrow represents up-regulation; red line shows down-regulation (Modified from Lallemend & Ernfors, 2012).*

### 1.2.1 Muscle Proprioceptive sensory neurons

Proprioception stems from the Latin word *proprius* meaning "one's own". In 1906, Charles Scott Sherrington described proprioception as the sixth sense corresponding to the sense of position and movement of the body parts (Sherrington, 1906). There are two types of proprioceptive sensory organs: the muscle spindle (MS) that provides information about changes in muscle length and the Golgi tendon organ (GTO) that detect changes in muscle tension. MSs are sensory organs encapsulated in the muscle mass. Within a MS, there are

several intrafusal fibers containing contractile proteins that change length with the muscle stretching. Once the MS stretches as the muscle lengthens, the dendrites of the PSNs wrapping the central region of the intrafusal fibers open mechanically gated ion channels triggering action potentials in the MS afferents. Unlike MSs, which are distributed in parallel with muscle fibers, GTOs are located in series with muscle fibers in the tendons, but the mode of activation of GTO-innervating PSNs afferents is similar to that of MS afferents (Purves et al., 2001).

Based on their innervation patterns, structure, and electrophysiological profile, PSNs can be segregated into three groups: type Ia and II afferents innervate deeply into the skeletal muscle through MSs, while type Ib afferents innervate GTOs at skeletal muscle joints. Each of those afferent types encode different information about the muscle state: type Ia responds to the rate of change in muscle length, velocity and are rapidly adapting, type II fires when the muscle is static and type Ib responds to muscle tension changes. In the spinal cord, the patterning of the central projections of PSNs is different depending on the afferent type. The Ia afferents project to specific interneurons but synapse directly to motor neurons in the ventral horn whereas type II and type Ib make synapses with interneurons at the intermediate zone (layer VII) of the spinal cord (Lallemend and Ernfors, 2012).

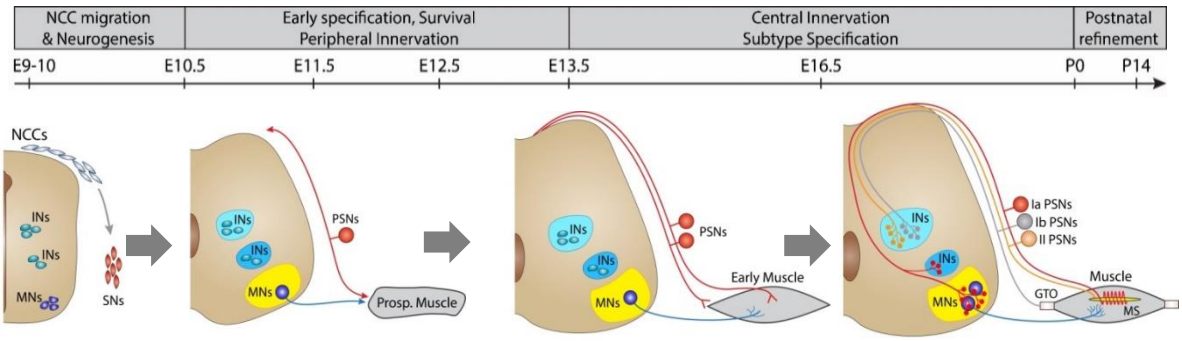


**Figure 3. Development of PSNs during mouse embryogenesis.**
*NCCs migration and neurogenesis of presumptive PSNs takes place at E9-E10. From E10.5 to E13.5, those early PSNs require early specification and only half of them survive the naturally occurring cell death period while they extend their peripheral projections. After peripheral innervation, different subtypes of PSNs will connect to either the interneurons pools (INs, types Ib and II afferents) and/or motoneurons (MNs, type Ia afferents) centrally in the spinal cord at around E17.*

The development of PSNs follows step-wised events characterized by the successive activation of various molecular pathways (see Figure 3). In mouse DRG, the earliest observation of presumptive PSNs is from E9.5. RUNX3 expression starts shortly after at E10.5, it specifies the PSNs population and reinforces the TRKC expression. Also, RUNX3

is important for PSNs to send axons to their peripheral targets, where NT3 is expressed (Kramer et al., 2006; Lallemend and Ernfors, 2012). At the same time, NT3-TRKC signaling is essential for cell survival during the developmental cell death period and for inducing the expression of the PSN specific transcription factor ER81 (Ernfors et al., 1994; Patel et al., 2003). Eventually, PSNs are characterized by the co-expression of TRKC, RUNX3 and ER81 during early embryonic development as well as by the expression of the calcium binding protein Parvalbumin (PV), TRKC and RUNX3 from E16.5 (Lallemend and Ernfors, 2012; de Nooij et al., 2013).

*Peripheral Innervation*

Peripheral innervation here refers to DRG axons that project peripherally to particular regions in the body. Focusing on PSNs peripheral innervation, they start to extend their peripheral axons towards their target as early as E10, forming the brachial plexus by E10.5. Subsequently, at limb levels, PSNs grow into developing limb (Hua et al., 2013) and will innervate fingers digits by E14. This axonal extension throughout the axial body levels is under the control of RUNX3 (Lallemend et al., 2012). During this early elongation period, NT3 secreted by the limb mesenchyme provides important trophic support to the axons of PSNs. NT3 from the peripheral target is essential primarily for the survival of PSNs before E13.5 (next Chapter) when the peripheral innervation occurs, but its role in the peripheral innervation process is still not clear (Patel et al., 2003). Nevertheless, it seems that MSs-derived NT3 is important for the functional maintenance of the connections between PSN afferents and MSs at later stages (Shneider et al., 2009). In addition, the NT3 signaling is required for the induction of ER81, which is necessary for the survival of particular subgroups of limb-innervating PSNs (de Nooij et al., 2013). It is noteworthy, however that at very early stages, the peripheral projections of PSNs grow together with the MNs axons (Wang et al., 2014) suggesting that MNs might also play a role in the guidance of PSNs peripheral projections. In support of this, fewer MSs were observed in limb muscles of mice devoid of MNs, while the number of PSNs was not affected (Poliak et al., 2016).

*Central innervation*

The central innervation corresponds to the axonal projections from DRG neurons that enter the spinal cord. This central projection starts after the peripheral innervation by DRG neurons has occurred. It has been shown that NT3 along with the two transcription factors RUNX3 and ER81 are important for the central innervation of PSNs. Knocking out any of

these factors lead to severe defects in the central projection of PSNs (Arber et al., 2000; Chen et al., 2006a; Inoue et al., 2002; Patel et al., 2003). However, the direct regulatory mechanisms that control the central innervation of PSNs are still not clear. Furthermore, NT3, ER81 and RUNX3 are involved in the peripheral innervation and survival of PSNs prior to central innervation (Lallemend et al., 2012; de Nooij et al., 2013; Patel et al., 2003). Since contact with the peripheral target has been proposed to play a major role in central innervation of PSNs (Wenner and Frank, 1995), it is difficult to rule out the possibility that the defects in the central innervation of PSNs observed in the absence of these factors might simply be a consequence of a lack or loss of peripheral innervation. Thus, conditional knockout of these key factors after peripheral innervation is completed is needed for dissecting out their direct functions in the central innervation.

### 1.2.2 Cutaneous Mechanoreceptive sensory neurons

Mechanoreceptive sensory neurons (MSNs) that innervate the skin to sense touch and vibration are called tactile mechanoreceptors. Cutaneous mechanoreceptors respond to mechanical stimuli such as pressure and vibration. There are four major types of tactile mechanoreceptors in mammalian skin based on their morphology: Merkel's disk, Meissner's corpuscle, Ruffini ending and Pacinian corpuscle. The first two are located within or beneath the epidermis, whereas the latter two are found much deeper within the subcutaneous tissue. They perceive different kinds of sensations and exhibit different rates of adaption. The Merkel and the Ruffini corpuscle end-organs are the slowly adapting type mechanoreceptors. Both of which produce a sustained response to static stimulation, although the Ruffini corpuscle responds to skin stretch as well (Johnson and Hsiao, 1992; Torebjök and Ochoa, 1980). The Meissner- and Pacinian corpuscles, on the other hand, are rapidly adapting mechanoreceptors that produce transient responses to stimulation. While Meissner corpuscles have small receptive fields that underlies the perception of flutter-vibration, the Pacinian corpuscles have large receptive fields that underlie the perception of high frequency vibration (Biswas et al., 2015; Talbot et al., 1968).

MSNs are the $RET^+$ and $TRKB^+$ DRG neurons generated from the first wave of neurogenesis. At later stages, these neurons diversify into rapidly adapting LTMR innervating Meissner and Pacinian corpuscles that participate in touch sensation. The central projection of these rapidly adapting LTMRs innervates layer III of the spinal cord. Disruption of RET signaling results in a complete loss of Pacinian corpuscles (Luo et al., 2009). At late embryonic stage, the $RET^+$ neurons start to express the transcription factor MAFA, which is

under the control of *c-MAF*. In *c-Maf* $^{-/-}$ mice, Pacinian corpuscles are severely atrophied at postnatal day (P) 0 (Wende et al., 2012). In *MafA* mutants, some LTMRs lose RET. Vice versa, in the *Ret* mutant, *MafA* expression is reduced (Bourane et al., 2009). Another lineage of MSNs are the TRKB$^{+}$/TRKC$^{-}$ neurons derived from the early neurogenesis, and they might be those innervating Merkel cells and Meissner corpuscles. SHOX2 plays a role in encoding this subtype of MSNs, where it is required to maintain TRKB expression and suppress TRKC (Abdo et al., 2011).

### 1.2.3 Nociceptive sensory neurons

Nociceptors are a group of DRG neurons that detect the noxious or potentially damaging stimuli that trigger pain sensation (Sherrington, 1906). Nociceptive sensory neurons (NSNs) usually have free nerve endings innervating the skin. Further, NSNs display two types of axons: myelinated Aδ fibers with fast transduction, which is associated with an initial intensive pain. The other is the unmyelinated, and thus slow conducting, C-fibers, which participate in a second phase of pain sensation (Purves et al., 2001).

The sensibility of each type of nociceptors is established by the expression of different ion channels with high threshold of activation, which allows responses to a particular stimulus, such as thermal, mechanical or chemical stimuli (Woolf and Ma, 2007).

During early development, NSNs are characterized by the expression of TRKA receptors. While the Aδ fibers are born together with PSNs and MSNs and do not express RUNX1, the majority of nociceptor (C-fibers) are born after E11.5 and express RUNX1 during embryogenesis (Marmigère and Ernfors, 2007). Around birth, NSNs are segregated into two major groups comprised of peptidergic and nonpeptidergic fibers. These two types of NSNs express different sets of receptors and ion channels. Further, they have different peripheral and central innervation patterns. Around half of the NSNs switch off TRKA expression and start expressing RET to become the nonpeptidergic nociceptors postnatally. Most of this group of NSNs binds to isolectin B4 (IB4). The other NSNs are peptidergic, which continue to express TRKA (some still co-expressing RET) without binding to IB4. The transcription factor RUNX1 seems to play an important role in this diversification as it is repressed in TRKA$^{+}$ peptidergic nociceptors at perinatal and postnatal development. In the RUNX1 conditional knockout mice, the transition of those NSNs from TRKA$^{+}$ to RET$^{+}$ is impaired (Chen et al., 2006b; Kramer et al., 2006; Woolf and Ma, 2007).

## 1.3 PROGRAMMED CELL DEATH DURING NEURONAL DEVELOPMENT

Apoptosis, also called programmed cell death (PCD), is a naturally occurring phenomenon during development that is necessary to build functional tissue and sculpt body parts. Throughout the development of the nervous system, one of the main functions of PCD is to control cell number in order to functionally integrate those cells into the environment and neuronal networks (Baehrecke, 2002).

In *C. elegans*, at least 12 genes has been identified to control the elimination of 131 cells out of a total of 1090 cells (Hengartner, 1999). The PCD in this model is typically intrinsically predetermined (Denaxa et al., 2018; Mi et al., 2018; Priya et al., 2018). In contrast, the natural cell death of DRG neurons is believed to be exclusively dependent on extrinsic cues from the peripheral targets where a limited amount of neurotrophic factors would be secreted. Retrograde transport of the survival signaling is activated when the axons of DRG neurons bind neurotrophins in their peripheral environment, leading to a competition between neurons for neurotrophins and thus for their survival (see next Chapter).

### 1.3.1 Neurotrophic theory

Upon the discovery of the neurotrophic factors, NGF, BDNF and NT-3 which are the high-affinity ligands for the TRKA, TRKB and TRKC receptors, respectively, the so-called neurotrophic theory was suggested, and in which the death of about 50% of DRG neurons is proposed to be due to the competition of those new born neurons for limiting amount of target-derived neurotrophic factors. According to the theory, only those neurons that randomly receive enough neurotrophins would survive during the natural cell death time window leading to a stochastic selection model. This competition happens when DRG neurons extend their projections within their peripheral tissue/target (Levi-Montalcini, 1987). In contrast, adult DRG neurons are no longer dependent on the neurotrophic factors for their survival (Bhattacharyya et al., 1997).

### 1.3.2 TRK signaling

The reason why early DRG neurons die without neurotrophin might not only be that the need of the neurotrophic receptor to bind its ligand in order to activate downstream survival-signaling pathway to counteract the mitochondrial death pathway, but also because the neurotrophic receptors could behave as a dependent receptor: in the presence of ligand, the receptor transduce a positive signal leading to survival, conversely, in the absence of ligand, the receptor initiates and sustains a signal for programmed cell death (Nikoletopoulou et al.,

2010). As shown in Figure 4, TRK receptors are phosphorylated upon binding with their ligand, and then activate both MEK/ERK and AKT survival signaling which eventually block proapoptotic BCL-2 homolog BAX (Bibel and Barde, 2000). Without binding with NT3, TRKC can release a pro-apoptotic fragment from a double-caspase cleavage, which leads to cell death through the intrinsic apoptotic pathway (Ichim et al., 2013). However, further experiments in the developing DRG will be however necessary to confirm this pathway.

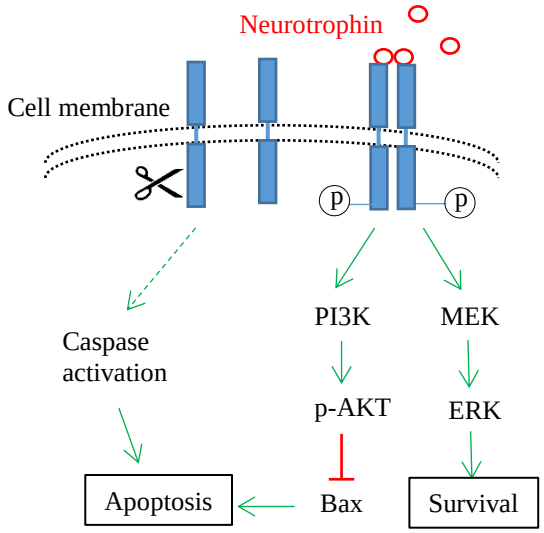


**Figure 4. TRK signaling.**
*Upon binding to neurotrophin dimers, the TRK receptor activates a downstream survival signaling pathway, which blocks the pro-apoptotic factor Bax. Without binding with neurotrophin, the intracellular region of TRK receptor might be cleaved and continuously lead to caspase activation. Lines ending in arrowheads indicate stimulation, whereas lines ending in bars indicate blockage. Dash line suggests a controversial pathway.*

Unlike TRK receptors that are restricted to specific neuronal types, another neurotrophic factor receptor p75, which has low affinity to all neurotrophic factors, is widely expressed in numerous cell types. All post-translational forms of the neurotrophin proteins, mature and pro-neurotrophins, can bind to p75 to not only promote survival but also induce apoptosis as it has a death domain while in contrast, only the mature form of neurotrophins bind TRK receptors to promote survival signaling. Interestingly, p75 can also interact with the TRKs receptors. For example, co-expression of p75 and TRKA can potentially lead to greater affinity to NGF, suggesting an additional regulation of TRK receptors for the neurotrophic factors (Chao et al., 1995; Pathak and Carter, 2017).

## 1.4 AXONAL GROWTH

Soon after neurogenesis, DRG neurons elongate their axons rapidly towards their targets due to a forward driven tension from the growth cone. Each neuronal growth cone comprises two domains: a central domain containing microtubules and a peripheral domain enriched with actin filaments. The actin from the peripheral domain continuously assembles at the front edge creating two structures called filopodia and lamellipodium, thus elongating the growth cone forward. At the same time, myosin-like motors drag the actin filament back towards the

central domain. Both forward and backward forces keep a balance leading to no net progress. Once the receptors from the surface of the growth cone binds to their respective ligands, the retrograde flow of actin filaments is suppressed, resulting in a shift toward the forward progress and then initiate or continue the axonal elongation (Goldberg, 2003). In order to project axons to the right targets, DRG neurons rely on both extrinsic cues and intrinsic factors during the elongation.

### 1.4.1 Extrinsic factors

Axonal guidance cues are molecules or proteins that if presented at the right time and place will either attract or repulse the growth cone (Song and Poo, 2001). For example, semaphorin 3A (SEMA3A, a short-range diffusible chemo-repellent guidance cue) and its receptor neuropilin-1 are required for mediating a chemo-repulsive signal for the DRG axons in the spinal cord (Masuda et al., 2003). SEMA3A is initially expressed across the entire spinal cord, however, as the axons of DRG neurons enter through the dorsal horn, SEMA3A expression is progressively downregulated (Fu et al., 2000). SEMA3A/Neuropilin-1 portray a neuron specific repelling mechanism in the developing ventral spinal cord as it repels $TRKA^+$ but not $TRKC^+$ neurons (Marmigère and Ernfors, 2007; Messersmith et al., 1995). Other short-range guidance cues that mediate chemo-repulsive activities for DRG axons are the cell adhesion molecules (CAMs) AXONIN 1/SC2 (Masuda et al., 2000). Two of the CAMs super families are involved in the guidance of neuronal specific projection of DRG axons in the gray matter of the spinal cord. AXONIN-1/TAG-1 mediate the NSNs to target to the dorsal horn whereas F11/F3/Contactin is required for the PSNs to connect with motoneurons in the ventral horn (Perrin et al., 2001).

### 1.4.2 Intrinsic factors

RUNX are proteins found to be involved in the early neuronal specific connectivity. As mentioned previously, RUNX1 is a transcription factor that is involved in the diversification of peptidergic as well as non-peptidergic neurons in the late embryonic stages and presumed to be involved in the central axonal projections of these two groups. $TRKA^+$ peptidergic neurons terminate their axons mainly in lamina I and the outer layer of lamina II in the spinal cord, whereas $TRKA^-$ non-peptidergic neurons innervate the inner lamina II. While the loss of RUNX1 results in the aberrant termination of non-peptidergic neurons to the outer layer of lamina II in mice (Chen et al., 2006b), its overexpression causes the peptidergic neurons to project ectopically to the inner lamina II (Kramer et al., 2006).

Similarly, for the PSNs population, changes in the transcription factor RUNX3 also have an effect on axonal extension and target innervation. As an example, forced expression of RUNX3 (normally expressed in $TRKC^+$ DRG neurons) in $TRKA^+$ DRG neurons alters the projection of the TRKA neurons so that they enter the spinal cord through the medial part of the dorsal funiculus, much like $TRKC^+$ axons do. Overexpression of RUNX3 can also cause type Ib and II proprioceptive axons (that normally terminate in the intermediate spinal cord) to shift to terminate to a more ventral position where type Ia central afferent project (Chen et al., 2006a). Taken together these results show that intrinsic factor, RUNX3, and its levels of expression can influence the central afferent patterning of DRG neurons.

# 2 RESULTS AND DISCUSSION

## 2.1 PAPER I

The sensation of pain is essential for preserving the functional integrity of our body. Yet the molecular mechanisms necessary to drive the development of pain-sensing neurons as well as to maintain their homeostasis in adult are still largely unknown. In this context, PRDM12, an epigenetic regulator belonging to the PRDM (PRDI-BF1 and RIZ homology domain) family of putative histone-methyltransferases (HMTs) (Hohenauer and Moore, 2012), has previously been reported to be essential in humans for pain sensitivity. Members of the PRDM family play multiple roles in developmental contexts, including neurogenesis, by driving and maintaining cell state transitions as well as by activating or repressing certain developmental signaling cascades (Matsukawa et al., 2015; Thelie et al., 2015). A recent report indicated that mutation of *PRDM12* can cause congenital insensitivity to pain (CIP), a rare phenotype in which the patient loses or have a malfunction of nociceptors from birth (Chen et al., 2015). However, the cellular and mechanistic insights on the causative deficits are missing.

In this paper, we demonstrated the specific expression pattern of PRDM12 in mouse DRG ranging from neurogenesis to the late embryonic stage using both RNA scope ® *in situ* hybridization and immunohistochemistry approaches. We observed that PRDM12 starts to express first in migrating $SOX10^+$ progenitor cells. After neurogenesis, we found that PRDM12 is exclusively expressed in $TRKA^+$ post-mitotic neurons while it was absent from the A-LTMRs lineages (TRKB, TRKC or RET). This expression pattern persists into adulthood.

We further studied the function of PRDM12 using *Prdm12* knockout mice. Although *Prdm12*$^{-/-}$ embryos did not show any morphological defects and survived until E18.5, we never observed any newborn mutant pups indicating perinatal lethality. Interestingly, we observed a complete loss of $TRKA^+$ neurons (both eTRKA and lTRKA population) in developing DRG leading to a smaller DRG size. In line with this, the total number of neurons was reduced by ~70% at E12.5 in *Prdm12*$^{-/-}$ DRG. However, the total number of A-LTMRs remained the same indicating that the absence of $TRKA^+$ is not compensated by them. We found that the loss of $TRKA^+$ neurons could be a result of reduced $SOX10^+$ precursor cells proliferation. We further confirmed the specific role of PRDM12 in the development of the nociceptive lineage by the absence in *Prdm12*$^{-/-}$ DRG of expression of nociceptor-specific markers at E18.5, including Substance P, calcitonin gene-related peptide (CGRP) and

Tyrosine hydroxylase (TH). The remaining neuronal populations in *Prdm12*$^{-/-}$ DRG are all myelinated and express either TRKC, TRKB or RET corresponding to the A-LTMRs.

We next evaluated whether the expression of PRDM12 was sufficient to drive the fate of nociceptive lineage in electroporated chicken embryos with plasmids expressing a FLAG-tagged PRDM12 or eGFP as a control. In contrast to the control, where eGFP traced cells were found in both glial cells and neurons that express TRKA or TRKC, we found that the FLAG-PRDM12$^{+}$ cells were restricted to neurons expressing TRKA. However, the percentage of TRKA$^{+}$ neurons remained the same in both FLAG-PRDM12$^{+}$ and eGFP, indicating that PRDM12 is sufficient to suppress TRKC but not induce TRKA$^{+}$ neurons.

In addition, we found that *Ngn1* required PRDM12 expression for its maintenance. However, *Prdm12* expression was not affected by neurogenins. In summary, we demonstrated that PRDM12 is necessary for neurogenesis and crucial for activation of the neurogenic program in NCCs that generate the whole nociceptive lineages (as shown in Figure 5).

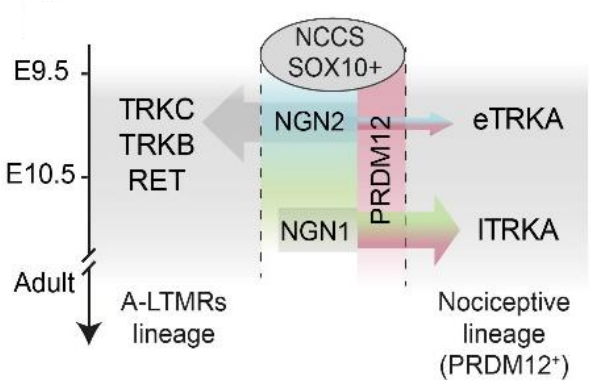


**Figure 5.** *Scheme recapitulating the role of PRDM12 in the commitment of neuronal precursor cells into the major different sensory neuron fate (unpublished figure).*

## 2.2 PAPER II

The neurons from the second wave of neurogenesis (lTRKA lineage) give rise to the majority of nociceptors, pruriceptors, and thermoreceptors of the adult DRG. In this lineage, RUNX1 starts to be express at E12.5 and plays a key role in the development and diversification of the populations in the lTRKA lineage (Chen et al., 2006b; Kramer et al., 2006). However, how RUNX1 expression is induced and regulated in these populations of DRG neurons has not previously been studied.

In this paper, we showed that the acquisition of RUNX1 was instructed by the early-born neurons derived from the first wave of neurogenesis in which almost all neurons express TRKC at early stages, prior to diversification. Using *TrkC*$^{cre}$*;Isl2*$^{DTA}$ mouse embryos to eliminate the early-born neurons before the induction of RUNX1 in lTRKA population, we

found that some neurons failed to induce RUNX1 and TRKA while the remaining $RUNX1^+$ population showed low levels of RUNX1 expression.

We screened for potential instructing molecules and found that fibroblast growth factor (FGF) and insulin-like growth factor (IGF) can induce RUNX1 in cultured DRG in a time-dependent manner that reflected the timing of RUNX1 induction *in vivo*. The induction of RUNX1 *in vitro* could be blocked by inhibiting either the MAPK or the PI3K pathway. We also found that FGF ligands (*Fgf1, Fgf* 2, *Fgf13, Fgf18*) were produced locally within the DRG whereas IGF ligands (*Igf* 1 and *Igf* 2) were expressed in the mesenchyme surrounding the DRG.

We observed an abundant expression of the fibroblast growth factor receptor 1 (FGFR1) in the DRG at the time of induction of RUNX1. We thus decided to analyze the $Wnt1^{Cre};Fgfr1^{fl/fl}$ mice (where *Fgfr1* is deleted in neural crest cells) to examine the requirement for FGFR1 signaling during development of $RUNX1^+$ neurons *in vivo*. At E12.5, these mice showed a decrease in the number of $ISL1^+$ neurons due to the decrease of $TRKA^+/RUNX1^+$ neurons. The intensity of RUNX1 in remaining $TRKA^+/RUNX1^+$ neurons was also reduced. However, this deficit was recovered at E14.5 when there was no difference between mutant and wild type indicating that other regulatory mechanisms might compensate for the early phenotype.

The partial reduction of RUNX1 expression in *Fgfr1* conditional knockout mice indicates that FGF signaling is one of several factors involved in the onset of RUNX1 expression in lTRKA neurons. It is also possible that IGF is one of the factors that is involved in the induction of RUNX1 and could compensate later for the loss of FGF signaling.

## 2.3 PAPER III

In this paper, we investigated the molecular mechanisms regulating the early cell death of PSNs. The traditional concept proposes that the cell death of the early born neurons in the DRG is due to the limited amount of neurotrophic factors secreted in the peripheral target, and suggests that the newborn neurons have equal capacity (and associated molecular traits) to compete for these neurotrophins. However, our data showed variability in expression of the receptor TRKC that could thus participate in mediating distinct competitiveness within neurons. We found that early single PSNs express different levels of TRKC before the cell death period starts (that is around E12). A correlation between the expression level of TRKC and phospho-AKT survival signaling suggested that higher TRKC expressing PSNs might have higher survival probability. To confirm this hypothesis, we challenged the capacity of

low TRKC neurons from *Runx3* mutant mice *in vitro* and found that they have lower survival rate. We also injected *TrkC*$^{CreERT2}$;*Ai14* mice with a low dose of 4-OHT, a catalyzed product of Tamoxifen, to trace the high TRKC expressing PSNs *in vivo*. The enrichment of those high TRKC expressing neurons in the total PSNs population after the cell death period directly demonstrated that high TRKC$^{+}$ PSNs have a higher probability to survive.

The setting of the different TRKC expression levels per cell is independent of its ligand NT3 so independent on the ligand availability but is intrinsically regulated by the transcription factor RUNX3. Interestingly, single-cell transcriptomic data revealed that PSNs can be clustered as two populations representing different cell fitness at E11.5. In line with this, one group expresses a high level of *Runx3* whereas the other group expresses low *Runx3*. The high *Runx3* expressing group is associated with more mature gene profiles before the cell death period. However, we showed that the difference in the expression profile of the two sub-populations is independent on the time of their birth.

Furthermore, we found that Retinoic Acid (RA) controls RUNX3 expression at a concentration-dependent manner *in vitro*. In our *Raldh2*$^{-/-}$ and conditional knock out mice, the deletion of enzymes that catalyze the production of RA causes a decrease in the expression of RUNX3 in PSNs during early developmental stage.

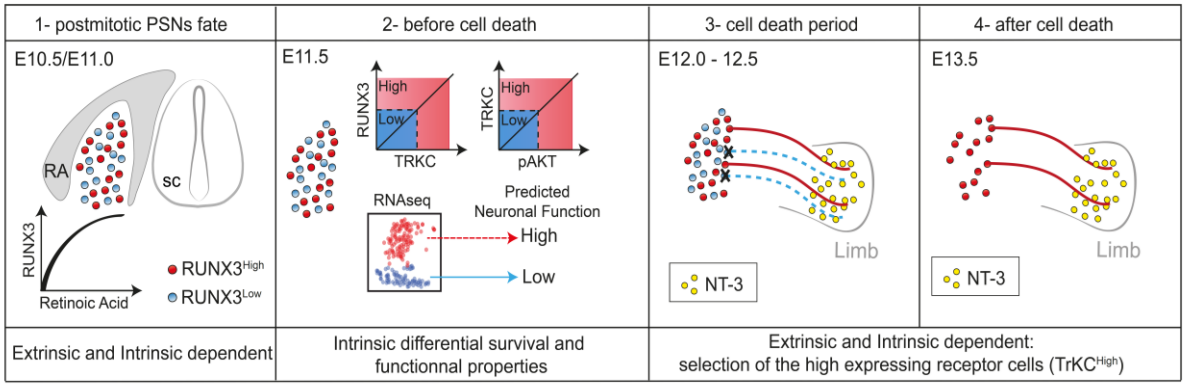


**Figure 6. Cell survival model of sensory neurons during development.**
*Proposed model for the selection of the TRKC neurons during development, in which neurons, soon after being generated, feature different functional molecular signatures that can predict either their survival or death during the cell death period (unpublished figure).*

Taken together, our findings propose an alternative model in which PSNs are genetically encoded with distinct intrinsic features that can participate in predicting their probability to survive during the cell death period (see Figure 6). We find that RA might instruct the differentiation of PSNs with differential capacities to integrate environmental cues for their

survival. In contrast to the classic competition model, this mechanism might provide an advantage to rapidly clear non-selected neurons during early development.

## 2.4 PAPER IV

In this paper, we investigated the mechanism underlying the axonal growth rate of DRG neurons during early development. We first found that the pioneering neurons growing towards limbs exhibit a faster axonal grow rate than the ones growing into the thoracic region of chicken embryos between the stage HHst25 and HHst27 grossly corresponding to E11 and E12 in mice. We also found that the level of RUNX3 expression displayed a positional difference along the rostro-caudal axis. There was a direct temporal correlation between the increase of RUNX3 and the increase of axon extension from the neurons that innervate the limbs region at abovementioned stage. Moreover, our data showed that the level of RUNX3 at different axis segment was independent on the peripheral target.

Next, we studied the functional role of RUNX3 by interfering with its activity. We electroporated chicken embryos with the dominant-negative construct RUNX1d, which inhibits the RUNX3 activity in DRG. We observed a remarkable decrease in axonal growth rate from brachial and lumbar DRG neurons with pCARUNX1d expression compared to controls. On the other hand, overexpression of RUNX3 by electroporating embryos with pCARUNX3 resulted in a two-fold increase in the axonal growth rate of thoracic neurons. We manipulated the level of electroporation efficiency by using another construct with a less strong promoter, pCMV. Electroporation of pCMVeGFP reduced the GFP expression levels by 60% compared to pCAeGFP, but the number of transfected neurons remain unchanged. Expression of pCMVRUNX1d led to a milder effect on axon growth compared to the pCARUNX1d-expressing neurons. These results suggested a graded activity of RUNX3 in influencing the axon growth rate of DRG neurons at different segmental levels.

We addressed the RUNX3 activity to determine the growth of DRG axons *in vivo* using mouse models. Similar to the experiments from chicken embryos, we observed a spatial difference in axonal growth in wild type mouse. In E11.5 *Runx3*$^{-/-}$, the axonal growth of brachial DRG neurons showed a significant decrease that was comparable to that of wild-type thoracic DRG *in vitro*. This result showed that the difference in axonal growth rate was abolished in the absence of RUNX3. We also found a similar defect *in vivo* using whole-mount immunofluorescent staining of *Runx3*$^{-/-}$*;Bax*$^{-/-}$*;Brn3a*$^{+/TLZ}$, which allowed visualization of all PSNs peripheral projections.

In the end, we screened for genes whose expression is dependent on the levels of RUNX3 expression and narrowed down to those related to axonal growth/cell migration. We found that one mechanism underlying the axon extension regulation by RUNX3 might be through inhibiting the Rho-kinase activity.

# 3 CONCLUSIONS AND PERSPECTIVES

How a group of homogeneous progenitors differentiates into distinct neuronal cell populations during the mammalian embryonic development is one of the most fundamental questions in developmental neurobiology. Unlike model organisms such as *C. elegans*, where predetermined patterning determinants guide the cell organization and pattern formation, mammalian embryos do not present strict and as robust pre-determination clues that would preclude the fate of any cell during early embryogenesis. Instead, progenitors in mammals are exposed to many factors during development, and must interpret both strong but also more subtle inductive molecules, which are generally co-expressed and both necessary for their fate specification. Ultimately, the correct integration of intrinsic and extrinsic molecules at specific time points and regions of the embryo enables progenitors to develop into the distinct and correct populations of cells on which the same principles of signal integration apply for further steps of development such as neuronal specification and axonal projection. Understanding this interplay of molecular regulation during development of the nervous system is still a major challenge in the field.

This thesis belongs within this context while investigating different aspects of events in the early development of somatosensory neurons. During neurogenesis, we discovered the crucial role of the epigenetic factor PRDM12 in driving the NCCs to nociceptive lineage fate. Although PRDM12 can repress the alternative lineage fate in post-mitotic cells, the fact that it in itself is insufficient to induce the nociceptive fate suggests that other regulatory molecules also operate in progenitors to control cell fate. Following neurogenesis, we found that the key transcriptional factor RUNX1 in the late-born neurons was regulated by factors secreted by the early-born neurons, supporting a concept that the early population also acts as pioneer neurons that influence later born populations.

Next, to study how the population size is controlled in the development of nervous system, we investigated the event of early cell death in PSNs, one of the early-born neuron populations. We found that there are two genetically encoded subgroups of PSNs with different survival capacity before the cell death period. One population with higher TRKC/RUNX3 expression, and consequently with a higher probability to survive, and a second population with low levels of TRKC/RUNX3 and predicted to have lower capacity to survive. This eventually brings up the question whether there is a competition amongst newborn PSNs as proposed in the classic neurotrophic theory. In other words, if the high TRKC population were to be eliminated before the cell death period, would the low TRKC

population survive? On the opposite, if all PSNs were forced to express the same levels of TRKC expression, will they all survive during the cell death period? Ongoing research in the lab will address these questions by manipulating the expression level of TRKC in PSNs before the cell death using two genetic mouse strategies: *TrkC*$^{creERT2/+}$*; ISL2*$^{DTA/DTA}$ (to kill the high TRKC neuron by a low dose of 4-OHT administration) and *TrkC*$^{creERT2/creERT2}$*;Rosa26*$^{PCATrkC}$ (since *TrkC*$^{creERT2}$ is a knock in, its homozygous form losses the endogenous TRKC), where all PSNs will be expected to express the same level of TRKC ectopically.

Finally, although the development of DRG neurons has been studied intensively in recent years, knowledge of the regulation of the central innervation of DRG neuron populations is still very limited. Yet, this last process during development is crucial as it defines whether sensory information is processed centrally or not, and correctly. While we found that RUNX3 plays a crucial role in determining the axonal growth rate of DRG neurons during peripheral innervation, unpublished data from the lab show that it also participates in the central innervation of PSNs in a muscle specific manner. Yet, future works will be needed to investigate in details the function of RUNX3 and of other factors in connecting peripheral DRG neurons to their central target by using a series of conditional knockout mouse lines for these factors after peripheral innervation is completed.

In summary, these findings help us to a better understanding of the molecular regulation operating during the development of the mammalian nervous system. In future studies, it would be of interest to examine whether these results also apply to humans. The fundamental knowledge of those molecular regulation events during the development will hopefully help improve treatment approaches for several diseases. For example, PRDM12 can be a good candidate target for patients with congenital insensitivity to pain. Another prospective is to improve cell therapies for neuronal replacement after cell loss in some neurological disorders. Our knowledge of the molecular heterogeneity, which affects survival capacity of the neurons, might provide an optimal and reproducible cell preparation for a successful transplantation.

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